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(11) EP 0 708 831 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant f the pat nt:
09.04.2003 Bulletin 2003/15

(21) Application number: **95918943.2**

(22) Date of filing: **03.05.1995**

(51) Int Cl.[7]: **C12N 15/55**, C12N 9/16, C12N 5/10, C07K 16/40, C07K 16/42

(86) International application number:
PCT/US95/05512

(87) International publication number:
WO 95/030008 (09.11.1995 Gazette 1995/48)

(54) Cell-density enhanced protein tyrosine phosphatases

Durch Zelldichte stimulierte Protein-Tyrosin-Phosphatasen

r .otéine-tyrosine-phosphatases stimulées par la densité cellulaire

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **03.05.1994 US 237940**

(43) Date of publication of application:
01.05.1996 Bulletin 1996/18

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D s ription

FIELD OF THE INVENTION

[0001] The present inv ntion relates gen rally to purified and isolated pr tein tyrosine phosphatas nzym s (PTPs) and polynucleotides encoding the same. PTPs of the inv ntion are characterized by upregulated mRNA transcription and/or translation, or p st-translational modification leading to incr ased total cellular enzyme activity as a function of increased cellular contact with neighboring cells. Such density enhanced PTPs are referred to as DEPTPs. An illustrative human Type III receptor-like density-enhanced protein tyrosine phosphatase has been designated huDEP-1.

BACKGROUND OF THE INVENTION

[0002] Protein tyrosine phosphorylation is an essential element in signal transduction pathways which control fundamental cellular processes including growth and differentiation, cell cycle progression, and cytoskeletal function. Briefly, the binding of growth factors, or other ligands, to a cognate receptor protein tyrosine kinase (PTK) triggers autophosphorylation of tyrosine residues in the receptor itself and phosphorylation of tyrosine residues in the enzyme's target substrates. Within the cell, tyrosine phosphorylation is a reversible process; the phosphorylation state of a particular tyrosine residue in a target substrate is governed by the coordinated action of both PTKs, catalyzing phosphorylation, and protein tyrosine phosphatases (PTPs), catalyzing dephosphorylation.

[0003] The PTPs are a large and diverse family of enzymes found ubiquitously in eukaryotes [Charbonneau and Tonks, *Ann.Rev. Cell Biol. 8*:463-493 (1993)]. Structural diversity within the PTP family arises primarily from variation in non-catalytic (potentially regulatory) sequences which are linked to one or more highly conserved catalytic domains. In general, soluble cytoplasmic PTP forms are termed non-receptor PTPs and those with at least one non-catalytic region that traverses the cell membrane are termed receptor-like PTPs (RPTPs).

[0004] A variety of non-receptor PTPs have been identified which characteristically possess a single catalytic domain flanked by non-catalytic sequences. Such non-catalytic sequences have been shown to include, among others, sequences homologous to cytoskeletal-associated proteins [Yang and Tonks, *Proc.Natl.Acad.Sci. (USA) 88*:5949-5953 (1991)] or to lipid binding proteins [Gu, *et al., Proc.Natl.Acad.Sci.(USA) 89*:2980-2984 (1992)], and/or sequences that mediate association of the enzyme with specific intracellular membranes [Frangioni *et al., Cell 68*:545-560 (1992)], suggesting that subcellular localization may play a significant role in regulation of PTP activity.

[0005] Analysis of non-catalytic domain sequences of RPTPs suggests their involvement in signal transduction mechanisms. However, binding of specific ligands to the extracellular segment of RPTPs has been characterized in only a few instances. For example, homophilic binding has been demonstrated between molecules of PTPμ [Brady-Kalnay, *et al., J. Cell. Biol.* 122:961-972 (1993)] *i.e.*, the ligand for PTPμ expressed on a cell surface is another PTPμ molecule on the surface of an adjacent cell. Little is otherwise known about ligands which specifically bind to, and modulate the activity of, the majority of RPTPs.

[0006] Many receptor-like PTPs comprise an intracellular carboxyl segment with two catalytic domains, a single transmembrane domain and an extracellular amino terminal segment [Krueger *et al., EMBO J. 9*:3241-3252 (1990)]. Subclasses of RPTPs are distinguished from one another on the basis of categories or "types" of extracellular domains [Fischer, et al., *Science 253*:401-406 (1991)]. Type I RPTPs have a large extracellular domain with multiple glycosylation sites and a conserved cysteine-rich region. CD45 is a typical Type I RPTP. The Type II RPTPs contain at least one amino terminal immunoglobulin (Ig)-like domain adjacent to multiple tandem fibronectin type III (FNIII)-like repeats. Similar repeated FNIII domains, believed to participate in protein:protein interactions, have been identified in receptors for IL2, IL4, IL6, GM-CSF, prolactin, erythropoietin and growth hormone [Patthy, *Cell 61*:13-14 (1992)]. The leukocyte common antigen-related PTP known as LAR exemplifies the Type II RPTP structure [Streuli *et al., J.Exp.Med. 168*: 1523-1530 (1988)], and, like other Type II RPTPs, contains an extracellular region reminiscent of the NCAM class of cellular adhesion molecules [Edelman and Crossin, *Ann.Rev.Biochem. 60*:155-190 (1991)]. The Type III RPTPs, such as HPTPβ [Krueger *et al., EMBO J. 9*:3241-3252 (1990)], contain only multiple tandem FNIII repeats in the extracellular domain. The Type IV RPTPs, for example RPTPα [Krueger *et al.* (1990) *supra*], have relatively short extracellular sequences lacking cysteine residues but containing multiple glycosylation sites. A fifth type of RPTP, exemplified by PTPγ [Barnes, *et al., Mol.Cell.Biol. 13*:1497-1506 (1993)] and PTPζ [Krueger and Saito, *Proc.Natl.Acad.Sci.(USA) 89*: 7417-7421 (1992)], is characterized by an extracellular domain containing a 280 amino acid segment which is homologous to carbonic anhydrase (CAH) but lacks essential histidine residues required for reversible hydration of carbon dioxide.

[0007] FNIII sequences characteristically found in the extracellular domains of Type II and Type III RPTPs comprise approximat ly nin ty amino acid r sidu s with a folding pattern similar to that observed for Ig-like d mains [Bork and Doolittl , *Proc.Natl.Acad.Sci(USA)* 89:8990-8994 (1992)]. Highly conserved FNIII sequences hav been identified in m re than fifty different eukaryotic and prokaryotic proteins [Bork and Doolittl , *Proc.Natl.Acad.Sci. (USA) 89*:

8990-8994 (1992)], but no g neralized function has been establish d for these domains. Fibronectin itself contains fifteen to seventeen FNIII domain sequences, and it has been demonstrated that the second FNIII domain ($FNIII_2$) contains a binding site for heparin sulphate proteoglycan [Schwarzbauer, *Curr.Opin.Cell Biol. 3*:786-791 (1991)] and that $FNIII_{13}$ and $FNIII_{14}$ ar responsibl for heparin binding through ionic interactions [Schwarzbauer, *Curr.Opin.Cell Biol. 3*:786-791 (1991)]. Perhaps the best characteriz d int raction for a fibronectin FNIII domain involves $FNIII_{10}$ which is th major site for cell adh sion [Ed lman and Crossin, *Ann.Rev.Biochem 60*:155-190 (1991); Leahy, *et al., Science 258*:987-991 (1992); Main, *et al., Cell* 71:671-678 (1992)]. $FNIII_{10}$ contains the amin acid s qu nc Arg-Gly-Asp (RGD) which is involved in promoting cellular adhesion through binding to particular members of the integrin superfamily of proteins.

[0008] Characteristics shared by both the soluble PTPs and the RPTPs include an absolute specificity for phosphotyrosine residues, a high affinity for substrate proteins, and a specific activity which is one to three orders of magnitude in excess of that of the PTKs *in vitro* [Fischer, *et al., Science 253*:401-406 (1991); Tonks, *Curr.Opin.Cell.Biol. 2*: 1114-1124 (1990)]. This latter characteristic suggests that PTP activity may exert an antagonistic influence on the action of PTKs *in vivo*, the balance between these two thus determining the level of intracellular tyrosine phosphorylation. Supporting a dominant physiological role for PTP activity is the observation that treatment of NRK-1 cells with vanadate, a potent inhibitor of PTP activity, resulted in enhanced levels of phosphotyrosine and generation of a transformed cellular morphology [Kiarlund, *Cell 41*:707-717 (1985)]. This observation implies potential therapeutic value for PTPs and agents which modulate PTP activity as indirect modifiers of PTK activity, and thus, levels of cellular phosphotyrosine.

[0009] Recent studies have highlighted aspects of the physiological importance of PTP activity. For example, mutations in the gene encoding a non-receptor hematopoietic cell protein tyrosine phosphatase, HCP, have been shown to result in severe immune dysfunction characteristic of the *motheaten* phenotype in mice [Schultz, *et al., Cell 73*: 1445-1454 (1993)]. Under normal conditions HCP may act as a suppressor of PTK-induced signaling pathways, for example, the CSF-1 receptor [Schultz, *et al., Cell 73*:1445-1454 (1993)). Some PTP enzymes may be the products of tumor suppressor genes and their mutation or deletion may contribute to the elevation in cellular phosphotyrosine associated with certain neoplasias [Brown-Shimer, *et al., Cancer Res. 52*:478-482 (1992); Wary, *el al., Cancer Res. 53*:1498-1502 (1993)]. Mutations observed in the gene for RPTPγ in murine L cells would be consistent with this hypothesis [Wary, *et al., Cancer Res. 53*:1498-1502 (1993)]. The observation that the receptor-like PTP CD45 is required for normal T cell receptor-induced signalling [Pingel and Thomas, *Cell 58*:1055-1065 (1989)] provides evidence implicating PTP activity as a positive mediator of cellular signalling responses.

[0010] Normal cells in culture exhibit contact inhibition of growth, *i.e.,* as adjacent cells in a confluent monolayer touch each other, their growth is inhibited [Stoker and Rubin, *Nature 215*:171-172 (1967)]. Since PTKs promote cell growth, PTP action may underlie mechanisms of growth inhibition. In Swiss mouse 3T3 cells, a phosphatase activity associated with membrane fractions is enhanced eight-fold in confluent cells harvested at high density as compared to cells harvested from low or medium density cultures [Fallen and Tong, *Proc.Natl.Acad.Sci. (USA) 88*:6996-7000 (1991)]. This elevated activity was not observed in subconfluent cell cultures brought to quiescence by serum deprivation. The enhanced phosphatase activity was attributed to a 37 kD protein, as determined by gel filtration, but was not otherwise characterized. Similarly, PTPs have been directly linked to density arrest of cell growth; treatment of NRK-1 cells with vanadate was able to overcome density dependent growth inhibition and stimulate anchorage independent proliferation, a characteristic unique to transformed, or immortalized, cells [Klarland, *Cell 41*:707-717 (1985); Rijksen, *et al., J. Cell Physiol. 154*:343-401 (1993)].

[0011] In contrast to these observations, PCT Publication No. WO 94/03610 discloses a transmembrane PTP, termed PTP35, the steady state mRNA level of which was observed to be at a maximum in actively growing cells. Little or no PTP35 mRNA expression was detected in confluent cell. This mode of regulation was also observed in mouse 3T3 cells. Thus, two RPTPs in the same cell type apparently participate in opposing processes, with one (PTP35) contributing to cellular growth and the other (the 35 kD PTP of Pallen and Tongs) contributing to cellular quiescence.

[0012] Interestingly, transcription of Type II RPTP LAR messenger RNA has been demonstrated to be upregulated in confluent fibroblast cell culture [Longo, *et al., J.Biol.Chem. 268*:26503-26511 (1993)]. LAR is proteolytically processed to generate a mature protein that is a complex of two non-covalently associated subunits, one containing the majority of the cell adhesion molecule-like extracellular domain [Yu, *et al., Oncogene 7*:1051-1057 (1992); Streuli, *et al., EMBO J. 11*:897-907 (1992)] and which is shed as cells approach confluence [Streuli, *et al., EMBO J. 11*:897-907 (1992)]. These observations lead to speculation regarding PTP involvement in modulation of cytoskeletal integrity, as well as other related cellular phenomena such as transformation, tumor invasion, metastasis, cell adhesion, and leukocyte movement along and passage through the endothelial cell layer in inflammation. The therapeutic implications are en rmous for modulators of PTP activity which are capable of regulating any or all of th s cellular ev nts.

[0013] Th re thus exists a ne d in the art to identify m mb rs f the PTP family of nzymes and to characterize th se prot ins in t rms of th ir amino acid and ncoding DNA s quences. Such information would provide for the large scale production of th pr teins, allow f r identificati n f cells which express the phosphatases naturally and permit

production of antibodies specifically reactive with the phosphatases. M reover, lucidation of th substrates, regulat ry mechanisms, and subc llular localization of these PTPs would contribute to an und rstanding of normal cell growth and provide information ess ntial for the development of therapeutic ag nts us ful for intervention in abnormal and/or malignant cell growth.

BRIEF DESCRIPTION OF THE INVENTION

[0014] As employed herein with respect to a protein tyrosine phosphatase, "density enhanced" denotes upregulated cellular mRNA transcription or translation and/or total cellular activity as a function of increased contact with neighboring cells.

[0015] According to the present invention, there is provided an isolated density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide of SEQ ID NO:2 or a variant thereof, wherein said variant is selected from:

(a) a human allelic variant or a variant from a heterologous species;

(b) a fragment thereof wherein:

(1) the fragment can be recognised by an antibody that is specific for a Type III receptor-like density enhanced protein tyrosine phosphatase, or

(2) at least one biological activity of a Type III receptor-like density enhanced phosphatase is retained or enhanced; said biological activity selected from

(i) binding to a substrate, ligand or counter-receptor of a Type III receptor-like density enhanced protein tyrosine phosphatase,

(ii) enzymatic activity of a Type III receptor-like density enhanced protein tyrosine phosphatase, or

(iii) signal transduction activity of a Type III receptor-like density enhanced protein tyrosine phosphatase;

(c) a variant thereof in which one amino acid is replaced and wherein

(1) the variant can be recognised by an antibody that is specific for a Type III receptor-like density enhanced protein tyrosine phosphatase, or

(2) at least one biological activity of a Type III receptor-like density enhanced phosphatase is retained or enhanced; said biological activity selected from

(i) binding to a substrate, ligand or counter-receptor of a Type III receptor-like density enhanced protein tyrosine phosphatase,

(ii) enzymatic activity of a Type III receptor-like density enhanced protein tyrosine phosphatase, or

(iii) signal transduction activity of a Type III receptor-like density enhanced protein tyrosine phosphatase; or

(3) a specific ligand/receptor binding or signalling function is disabled; or

(d) a mutant thereof in which a cysteine residue in the catalytic domain is substituted with a serine residue.

[0016] In another aspect, the present invention discloses purified and isolated polynucleotides (*e.g.*, DNA and RNA transcripts, both sense and anti-sense strands) encoding a Type III density enhanced receptor-like protein tyrosine phosphatase enzymatic activity exemplified by the human phosphatase huDEP-1 and variants, including fragments, thereof (*i.e.*, fragments and deletion, addition or substitution analogs) which possess binding and/or immunological properties inherent to Type III density enhanced phosphatases. According to this aspect of the invention, there is provided a polynucleotide encoding a density enhanced Type III receptor -like protein tyrosine phosphatase polypeptide, or variant of the invention. Preferr d DNA molecules f the inv ntion include cDNA, g nomic DNA and wholly or partially ch mically synth sized DNA molecul s. A presently pref rred polynucleotide is the DNA as set forth in SEQ ID NO: 1, encoding the human DEP-1 p lypeptide of SEQ ID NO: 2. Also provided are recombinant plasmid and viral

DNA constructions (expression constructs) which include sequences encoding Type III density enhanced receptor-like protein tyrosine phosphatase polypeptides or variants of the invention, especially constructions wherein the Type III density enhanced phosphatase encoding sequence is operatively linked to a homologous or heterologous transcriptional regulatory element or elements.

[0017] According to this aspect of the invention, there is also provided a purified and isolated polynucleotide selected from the group consisting of (a) the DNA sequence set at in SEQ ID NO: 1, and (b) a DNA molecule which hybridises under stringent conditions to the protein coding portion of the DNA of (a)

[0018] As another aspect of the invention, prokaryotic or eukaryotic host cells transformed or transfected with DNA sequences of the invention are provided which express a Type III density enhanced receptor-like protein tyrosine phosphatase polypeptide or variants thereof. Host cells of the invention are particularly useful for large scale production of Type III density enhanced phosphatase polypeptides, which can be isolated from either the host cell itself or the medium in which the host cell is grown. Host cells which express Type III density enhanced phosphatase polypeptides on the extracellular membrane surface are also useful as immunogens in the production of anti-Type III density enhanced phosphatase antibodies.

[0019] Also disclosed are purified and isolated Type III density enhanced phosphatase polypeptides, including fragments and variants thereof. A preferred Type III density enhanced phosphatase polypeptide is set forth in SEQ ID NO: 2. Novel Type III density enhanced phosphatase polypeptides and variant polypeptides may be obtained as isolates from natural sources, but are preferably produced by recombinant procedures involving host cells of the invention. Completely glycosylated, partially glycosylated and wholly un-glycosylated forms of the Type III density enhanced phosphatase polypeptide may be generated by varying the host cell selected for recombinant production and/or post-isolation processing. Variant Type III density enhanced phosphatase polypeptides of the invention may comprise water soluble and insoluble polypeptides including analogs wherein one or more of the amino acids are deleted or replaced: (1) without loss, and preferably with enhancement, of one or more biological activities or immunological characteristics specific for Type III density enhanced phosphatases; or (2) with specific disablement of a particular ligand/receptor binding or signalling function.

[0020] Also disclosed are peptides, polypeptides, and other non-peptide molecules which specifically bind to Type III density enhanced phosphatases of the invention. Preferred binding molecules include antibodies (*e.g.*, monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, anti-idiotype antibodies, CDR-grafted antibodies and the like), counterreceptors (*e.g.*, membrane-associated and soluble forms) and other ligands (*e.g.*, naturally occurring or synthetic molecules), including those which competitively bind Type III density enhanced phosphatases in the presence of anti-Type III density enhanced phosphatase monoclonal antibodies and/or specific counterreceptors. Binding molecules are useful for purification of Type III density enhanced phosphatase polypeptides of the invention and for identifying cell types which express the polypeptide. Binding molecules are also useful for modulating (*i.e.*, inhibiting, blocking or stimulating) the *in vivo* binding and/or signal transduction activities of Type III density enhanced phosphatases.

[0021] According to this aspect of the invention, there is provided an antibody capable of specifically binding to a density enhanced type III receptor-like protein tyrosine phosphatase polypeptide or vaiant of the invention. Also provided are hybridoma cell lines which produce antibodies specific for Type III density enhanced receptor-like protein tyresine phosphatase polypeptides or variants of the invention. Techniques for producing hybridomas which secrete monoclonal antibodies are well known in the art. Hybridoma cell lines may be generated after immunizing an animal with a purified Type III density enhanced phosphatase, or variants thereof, or cells which express a Type III density enhanced phosphatase or a variant thereof on the extracellular membrane surface. Immunogen cell types include cells which express a Type III density enhanced phosphatase *in vivo*, or transfected or transformed prokaryotic or eukaryotic host cells which normally do not express the protein *in vivo*.

[0022] The value of the information contributed through the disclosure of the DNA and amino acid sequences of human DEP-1 is manifest. In one series of examples, the disclosed human DEP-1 cDNA sequence makes possible the isolation of the human DEP-1 genomic DNA sequence, including transcriptional control elements. Transcriptional control elements comprehended by the invention include, for example, promoter elements and enhancer elements, as well as elements which contribute to repression, or downregulation, of mRNA transcription. Control elements of this type may be 5' DNA sequences or 3' DNA sequences with respect to the protein-encoding structural gene sequences, and/or DNA sequences located in introns. The 5' and/or 3' control elements may be proximal and/or distal the protein-encoding sequences of the structural gene. Identification of DNA sequences which modulate mRNA transcription in turn permits the identification of agents which are capable of effecting transcriptional modulation.

[0023] In another aspect, identification of polynucleotides encoding other Type III density enhanced phosphatases, huDEP-1 allelic variants and heterologous species (*e.g.*, rat or mouse) DNAs is also comprehended. Isolation of the huDEP-1 genomic DNA and heterologous species DNAs may be accomplished by standard nucleic acid hybridization techniques, under appropriately stringent conditions, using all or part of the DEP-1 DNA or RNA sequence as a probe to screen an appropriate library. Alternatively, polymerase chain reaction (PCR) using oligonucleotide primers that are

designed based on the known nucleotide sequence can be used to amplify and identify other cDNA and g nomic DNA sequences. Synthetic DNAs encoding Type III density enhanced phosphatase polypeptide, including fragments and other variants thereof, may b synthesized by conventional methods.

[0024] DNA sequence information of th invention also makes possible the dev lopm nt, by homologous recombination or "knockout" strat gies [se , *e.g.*, Capecchi, *Science 244*:1288-1292 (1989)], of rodents that fail to xpr ss a functional Type III density enhanced phosphatase polypeptide or that xpress a variant Typ III d nsity enhanced phosphatas polypeptid . Such rodents are useful as models for studying the activities of Typ III density nhanced phosphatases and modulators thereof *in vivo.*

[0025] DNA and amino acid sequences of the invention also make possible the analysis of Type III density enhanced phosphatase regions which actively participate in counterreceptor binding, as well as sequences which may regulate, rather than actively participate in, binding. Identification of motifs which participate in transmembrane signal transduction is also comprehended.

[0026] Also comprehended is identification of motifs which determine subcellular localization of the immature and mature Type III density enhanced phosphatase proteins.

[0027] DNA of the invention is also useful for the detection of cell types which express Type III density enhanced phosphatase polypeptides. Identification of such cell types may have significant ramifications for development of therapeutic and prophylactic agents. Standard nucleic acid hybridization techniques which utilize *e.g.*, huDEP-1 DNA to detect corresponding RNAs, may be used to determine the constitutive level of Type III density enhanced phosphatase transcription within a cell as well as changes in the level of transcription in response to internal or external agents. Identification of agents which modify transcription, translation and/or activity of Type III density enhanced phosphatases can, in turn, be assessed for potential therapeutic or prophylactic value. DNA of the invention also makes possible *in situ* hybridisation of *e.g.* huDEP-1 DNA to cellular RNA, to determine the cellular localisation of Type III density enhanced phosphatase specific messages within complex cell populations and tissues.

[0028] Polynucleotides of the present invention also provide a method whereby substrate or other molecules which interact with Type III density enhanced phosphatases can be identified. According to this aspect of the present invention, there is provided a method for isolating a polynucleotide encoding a polypeptide that binds to a density enhanced. Type III receptor-like protein tyrosine phosphatase polypeptide or variant of the invention comprising the steps of (a) transforming or transfecting host cells with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA-binding domain and an activating domain; (b) expressing in the host cells a first hybrid DNA sequence encoding a first fusion of part or all of a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide or variant of the invention and either the DNA-binding domain or the activating domain of the transcription factor; (c) expressing in the host cells a library of second hybrid DNA sequences encoding second fusions of part or all of putative density enhanced Type III receptor-like protein tyrosine phosphatase binding polypeptides and either the activating domain or DNA binding domain of the transcription factor which is not incorporated in the first fusion; (d) transforming or transfecting the host cells with a DNA construct comprising a protein tyrosine kinase gene; (e) detecting binding of density enhanced Type III receptor-like protein tyrosine phosphatase binding polypeptide(s) to the phosphatase polypeptide in the host cell by detecting the production of reporter gene product in the host cell(s); and (f) isolating the second hybrid DNA sequences encoding the phosphatase binding polypeptide or variant from the host cell(s).

[0029] A presently preferred method for identifying interacting molecules comprises the steps of: a) transforming or transfecting appropriate host cells with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA-binding domain and an activating domain; b) an optional step of cotranforming or co-transfecting the same host cells with a protein tyrosine kinase (e.g., v-src, c-src or the like) in order to phosphorylate potential interacting components and/or substrates introduced as in (d) below; c) expressing in the host cells a first hybrid DNA sequence encoding a first fusion of part or all of *e.g.*, a huDEP-1 isoform and either the DNA-binding domain or the activating domain of the transcription factor; d) expressing in the host cells a library of second hybrid DNA sequences encoding second fusions of part or all of putative DEP-1 isoform-binding proteins and either the activating domain or DNA binding domain of the transcription factor which is not incorporated in the first fusion; e) detecting binding of DEP-1 isoform-binding proteins to the DEP-1 isoform in a particular host cell by detecting the production of reporter gene product in the host cell; and f) isolating second hybrid DNA sequences encoding DEP-1 isoform-binding protein from the particular host cell. Variations of the method altering the order in which *e.g.*, the huDEP-1 isoforms and putative huDEP-1 isoform-binding proteins are fused to transcription factor domains, either at the amino terminal or carboxy terminal end of the transcription factor domains, are contemplated. In a preferred method, the promoter is the ADH1 promoter, the DNA-binding domain is the *lexA* DNA-binding domain, the activating domain is the GAL4 transactivation domain, the reporter gene is the *lacZ* gene and the host cell is a y ast host cell. Most pref rably, th promotes is the beta-galactosidase promot r, th DNA-binding domain is the *lexA* DNA-binding domain, the achivaring domain is the GAL4 transactivation domain, the repore gene is the *lacz* g ne and th host cells are yeast host cells. Those f ordinary skill in the art will readily envision that any of a number of oth r r porter genes and

host cells ar easily amenable to this technique. Likewise, any of a number of transcription factors with distinct DNA binding and activating domains can be utilized in this procedure, either with both the DNA binding and activating domains derived from the same transcription factor, or from different, but compatible transcription factors. As another variation of this method, mutant DEP-1 polypeptides, wherein a cyst in residu in the catalytic domain has b en substitut d with a serine residu , can be mploy d in this technique. Mutations of this typ hav be n demonstrated with other phosphatases to recognize and bind substrat s, but do not d phosphorylat the substrate since th phosphatase is inactive as a result of th mutation.

[0030] An alternative identification method contemplated by the invention for detecting proteins which bind to a Type III density enhanced receptor-like protein typosine phosphatase polypeptide isoform of the invention comprises the steps of: a) transforming or transfecting appropriate host cells with a hybrid DNA sequence encoding a fusion between a putative Type III density enhanced phosphatase isoform-binding protein and a ligand capable of high affinity binding to a specific counterreceptor; b) expressing the hybrid DNA sequence in the host cells under appropriate conditions; c) immobilizing fusion protein expressed by the host cells by exposing the fusion protein to the specific counterreceptor in immobilized form; d) contacting the Type III density enhanced phosphatase isoform with the immobilized fusion protein; and e) detecting the Type III density enhanced phosphatase isoform bound to the fusion protein using a reagent specific for the Type III density enhanced phosphatase isoform. Presently preferred ligands/counterreceptor combinations for practice of the method are glutathione-S-transferase/glutathione, hemagglutinin/hemagglutinin-specific antibody, polyhistidine/nickel and maltose-binding protein/amylose.

[0031] Additional methods to identify proteins which specifically interact with Type III density enhanced phosphatase (*i.e.*, substrates, ligands, modulators, *etc.*) are also contemplated. In one example, purified and isolated Type III density enhanced phosphatase polypeptide (*e.g.*, huDEP-1 polypeptide) can be covalently coupled to an immobilized support (*i.e.*, column resins, beads, *etc.*) and incubated with cell lysates to permit protein/protein interactions. Proteins which interact with the immobilized DEP-1 polypeptide can then be eluted from the support with gradient washing techniques which are standard in the art.

[0032] As another example, protein overlay techniques can be employed. DNA from cells which either express *e.g.*, huDEP-1 or express polypeptides which can modulated or bind to huDEP-1, can be isolated and a library constructed by standard methods. This library can then be expressed in a heterologous cell line and resulting colonies transferred to an immobilizing support. Expressed proteins from these colonies are then contacted with DEP-1 and incubated under appropriate conditions to permit DEP-1/protein interactions. The resulting Type III density enhanced phosphatase/protein complexes formed can be detected by incubation with a specific Type III density enhanced phosphatase antibody. Colonies which interact with the specific antibody contain DNA encoding a protein which interacts with the Type III density enhanced phosphatase. Alternatively, cell or tissue lysates may be employed in this technique, using cells or tissues which normally express DEP-1, or cells which have been previously transfected or transformed with DEP-1 encoding DNA.

BRIEF DESCRIPTION OF THE DRAWING

[0033] Numerous other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description thereof, reference being made to the drawing wherein:

Figures 1A through 1B are photographs of Northern blot analysis autoradiograms; and
Figures 2 shows the density-dependent expression of DEP-1.

DETAILED DESCRIPTION OF THE INVENTION

[0034] The present invention is illustrated by the following examples relating to the isolation and characterization of genes encoding Type III density enhanced phosphatase polypeptides. Example 1 relates to the isolation of cDNA encoding human DEP-1. Example 2 discusses the tissue distribution of huDEP-1 as determined by Northern blot analysis. Example 3 addresses the generation of antibodies specific for DEP-1 and fragments thereof. Example 4 demonstrates expression of a huDEP-1 cDNA clone in COS cells. Example 5 relates to detection of endogenous expression of huDEP-1 in fibroblast cells. Example 6 addresses expression of huDEP-1 as a function of cell culture density. Example 7 relates to identification of ligands of huDEP-1. Example 8 discusses identification of modulators and substrates of huDEP-1 activity. Example 9 details characterization of the genomic huDEP-1 DNA.

Exampl 1

Isolation and Charact rization of huDEP-1 DNA

[0035] In initial fforts to isolate cDNA encoding a novel human phosphatas regulated by a c ll density-dependent mechanism, PCR prim rs w re synthesized based on conserv d amino acid sequences common to many previously identified phosphatases. These primers w re then used to amplify polynucleotides from a cDNA library, th resulting amplification products were sequenced, and these sequences compared to previously reported DNA sequences.

[0036] Degenerate primers, corresponding to conserved PTP amino acid sequences set out in SEQ ID NO: 3 and SEQ ID NO: 4, were synthesized and used to prime a PCR with a HeLa cell cDNA library as template.

KCAQYWP SEQ ID NO: 3

HCSAGIG SEQ ID NO: 4

The corresponding primers used in the PCR reaction are set forth in SEQ ID NO: 5 and SEQ ID NO: 6, respectively, employing nucleotide symbols according to 37 U.S.C. § 1.882.

5´-AARTGYGCNCARTAYTGGCC-3´ SEQ ID NO: 5

3´-GTRACRTCRCGNCCITADCC-5´ SEQ ID NO: 6

Sequencing of seventy-seven independent subclones revealed seven distinct sequences, six of which corresponded to PTPs for which DNA sequences had previously been published, and included PTP1B [Tonks, *et al., J.Biol.Chem* 263:6722-6730 (1988)], TCPTP [Cool, *et al., Proc.Natl.Acad.Sci(USA) 86*:5257-5261 (1989)], RPTPα [Krueger, *et al., EMBO J. 9*:3241-3252 (1990)], LAR [Streuli, *et al., J.Exp.Med. 168*:1523-1530 (1988)], PTPH1 [Yang and Tonks, *Proc. Natl.Acad.Sci. (USA) 88*:5949-5953 (1991)], and PTPμ [Gebbink, *et al., FEBS Lett. 290*:123-130 (1991)]. The seventh clone was determined to comprise a unique 300 bp PCR fragment and was used to screen an oligo-dT-primed HeLa cell cDNA library (Stratagene, La Jolla, CA) in an effort to isolate a corresponding full-length cDNA. Approximately 1.8 x 10^6 phage plaques were screened as previously described [Yang and Tonks, *Proc.Natl.Acad.Sci.(USA)* 88:5949-5953 (1991)] and twenty-four positive clones were identified. The largest insert, a 5.1 kb cDNA, was cloned into pUC119, sequenced by the dideoxy chain termination method, and found to contain an open reading frame of 4011 nucleotides encoding a novel receptor-like PTP of 1337 amino acids. The DNA sequence of the 5.1 kb insert is set out in SEQ ID NO: 1, and its predicted amino acid sequence is set out in SEQ ID NO: 2. This human density-enhanced PTP was designated huDEP-1.

[0037] The proposed initiating ATG codon of the huDEP-1 gene is flanked by a purine (G) at the -3 position and is thus in agreement with the Kozak rules for initiation [Kozak, J. *Cell Biol. 108*:229-241 (1989)]. There is an in-frame stop codon approximately 290 bp upstream of the predicted initiation site, and the initiating ATG is followed by a hydrophobic region that may serve as a signal sequence. Based on the statistical analysis of known cleavage sites for the signal peptidase [von Heijne, *Nuc.Acids Res. 14*:4683-4690 (1986)], the amino terminus of the mature huDEP-1 polypeptide is assigned to Gly^{37}. A second hydrophobic region is found between amino acids 977 and 996, and is followed by a stretch of predominantly basic residues, characteristic of a stop transfer sequence. Therefore, an extracellular region of 940 amino acids and an intracellular portion of 341 amino acids are predicted for the mature hUDEP-1 protein. The extracellular domain comprises eight FNIII domains, and thirty-three potential sites for N-linked glycosylation are predicted. Thus, huDEP-1 conforms to the RPTP Type III topography according to the nomenclature of Fischer *et al., supra.* Unlike most RPTPs which possess a tandem repeat of catalytic domains, the cytoplasmic region contains a single catalytic domain spanning amino acid residues 1060 through 1296. Human DEP-1 is therefore representative of an expanding group of RPTPs with a single catalytic domain that includes PTPβ [Krueger, *et al., EMBO J. 9*:3241-3252 (1990)], DPTPIOD of *Drosophila* [Tian, *et al., Cell 76*:675-685 (1991); Yang, *et al., Cell 67*:661-673 (1991)], DPTP4E of *Drosophila* [Oon, *et al., J.Biol.Chem. 268*:23964-23971 (1993)], and the recently described SAP-l enzym [Matozaki, *et al., J.Biol.Chem. 269*:2075-2081 (1994)]. Amino acid sequence comparison of the catalytic domain of huDEP-1 with other PTP domains r veal d huDEP-1 is most cl s ly related t PTPβ and SAP-1. The s quenc includes s veral Ser-Pro motifs, as well as potential sites for phosphorylation by cas in kinase II.

Example 2

Northern Analysis of huDEP-1 Tissue Distribution

[0038] Because the expression of PTPs has previously been demonstrated to be ubiquitous in eukaryotes, various human tissues were analyzed in order to determine the relative degree of huDEP-1 mRNA expression.

[0039] RNA Multi Tissue Northern blot filters (Clontech, Palo Alto, CA), containing immobilized RNA from various human tissues, were probed with a 1.6 kb *Hind*III/*Eco*RI fragment of the huDEP-1 cDNA previously radiolabeled to a specific activity of 1.5 X 10^6 cpm/ng using a Megaprime DNA labeling kit (Amersham, Arlington Heights, IL). This probe represented the entire length of the isolated huDEP-1 cDNA. Hybridization was performed for 16 hours at 65°C in a hybridization buffer containing 0.5 M Na_2HPO_4, 7% SDS, 1 mM EDTA, and labeled probe at a concentration of 10^6 cpm/ml. Filters were then washed 5 times at 65°C in 40 mM Na_2HPO_4, 1% SDS, and 1 mM EDTA. The membrane was then subjected to autoradiography. The results are presented in Figures 1A and 1B, wherein the human tissue source of immobilized RNA is as follows. In Figure 1A, RNA in lane 2 is from heart, lane 3 from brian, lane 4 from placenta, lane 5 from lung, lane 6 from liver, lane 7 from skeletal muscle, lane 8 from kidney, and lane 9 from pancreas. In Figure 1B, RNA in lane 2 is from spleen, lane 3 from thymus, lane 4 from prostrate, lane 5 from testis, lane 6 from ovary, lane 7 from small intestines, lane 8 from colon, and lane 9 from peripheral blood leukocyte.

[0040] Northern analysis indicated that huDEP-1 is expressed in most tissues analyzed, with particularly high mRNA levels detected in placenta, kidney, spleen and peripheral blood leukocytes.

Example 3

Generation of huDEP-1 Polyclonal Antibodies

[0041] Two peptides, unique to huDEP-1 and corresponding to amino acid residues 1297 through 1315 and residues 1321 through 1334 in SEQ ID NO: 2 (downstream from the catalytic region) were synthesized with an additional amino terminal cysteine residue and conjugated to rabbit serum albumin (RSA) with *m*-maleimido benzoic acid N-hydroxy-succinimide ester (MBS)(Pierce, Rockford, IL). Immunization protocols with these peptides were performed by Cocalico Biologicals (Reamstown, PA). Initially, a pre-bleed of the rabbits was performed prior to immunization. The first immunization included Freund's complete adjuvant and 500μg conjugated peptide or 100 μg purified peptide. All subsequent immunizations, performed four weeks after the previous injection, included Freund's incomplete adjuvant with the same amount of protein. Bleeds were conducted seven to ten days after the immunizations.

[0042] For affinity purification of the antibodies, huDEP-1 peptide conjugated to RSA with MBS, was coupled to CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden). Antiserum was diluted 10-fold in 10 mM Tris-HCl, pH 7.5, and incubated overnight with the affinity matrix. After washing, bound antibodies were eluted from the resin with 100 mM glycine, pH 2.5.

[0043] The antibody generated against conjugated amino acid residues 1297 through 1315 was designated anti-CSH-241, and the antibody raised against the conjugated peptide corresponding to amino acid residues 1321 through 1334 was designated anti-CSH-243.

Example 4

Expression of huDEP-1 by Transfected Host Cells

[0044] To study the protein product of the huDEP-1 cDNA, the 5.1 kb *Eco*RI insert was cloned into the expression vector pMT2 [Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989) pp 16.17-16.22] and transfected into COS cells grown in DMEM supplemented with 10% FCS. Transfections were performed employing calcium phosphate techniques [Sambrook, et al (1989) pp. 16.32-16.40, *supra*] and cell lysates were prepared forty-eight hours after transfection from both transfected and untransfected COS cells. Lysates were subjected to analysis by immunoblotting using anti-CSH-243 antibody, and PTP assays of immune complexes as addressed below.

[0045] In immunoblotting experiments, preparation of cell lysates and electrophoresis were performed. Protein concentration was determined using BioRad protein assay solutions. After semi-dry electrophoretic transfer to nitro-cellulose, the membranes were blocked in 500 mM NaCl, 20 mM Tris, pH 7.5, 0.05% Tween-20 (TTBS) with 5% dry milk. After washing in TTBS and incubation with secondary antibodies (Amersham), enhanced chemiluminescence (ECL) protocols (Amersham) were performed as described by the manufacturer to facilitate detection.

[0046] For immune complex PTP assays, 60 μg of cell lysate were immunoprecipitated with 20 μl of anti-CSH-243 antisera or preimmune rabbit serum bound to 25 μl of Protein-A Sepharose (Pharmacia). After overnight incubation at

4° C, the immune complexes were washed three times in washing buffer (1% Triton X-100, 150 mM NaCl, 20 mM Hepes, pH 7.5, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 1 mM benzamidine, and 1 mM DTT) and once in assay buffer (25 mM imidazole, pH 7.2, 0.5 mg/ml BSA, and 1 mM DTT). Protein-A Sepharose immune complexes were then resuspended in 150 μl of assay buffer and assayed for PTP activity as triplicates. Assays were performed for 6 minutes at 30° C in a total volume of 60 μl using 3 μM [^{32}P-Tyr]-reduced carboxymethylated (RCM) lysozyme as substrate [Flint, *et al.*, *EMBO J. 12*:1937-1946 (1993)].

[0047] Affinity-purified anti-CSH-243 antibodies specifically detected a protein of 180 kD molecular weight in lysates from transfected cells. Furthermore, when immune complexes were analyzed for PTP activity, almost 10-fold higher activity was detected in anti-CSH-243 immune complexes from the transfected cells compared to the untransfected cells. This PTP activity was largely absent in immune complexes derived from immunoprecipitations with blocked antiserum or preimmune serum. It was concluded that the hUDEP-1 cDNA encodes a 180 kD protein with intrinsic PTP activity.

Example 5

Endogenous Expression of huDEP-1

[0048] To characterize endogenously expressed huDEP-1, lysates from different cell lines including CEM (ATCC CCL 119), HeLa (ATCC CCL 2), 293 (ATCC CRL 1573), Jurkat (ATCC TIB 152), K562 (ATCC CCL243), HL60 (ATCC CCL 240), WI38 (ATCC CCL 75) and AG 1518 (Coriell Cell Repositories, Camden, NJ) were analyzed by immunoblotting with antibody anti-CSH-243 as described in Example 4.

[0049] WI38 cells, a diploid fetal lung fibroblast-like cell line with finite life span, showed the highest expression. Similar levels of expression were also detected in AG 1518 foreskin fibroblast cells.

[0050] To further examine the expression of huDEP-1, lysates from metabolically labeled cells were analyzed by immunoprecipitation and SDS-gel electrophoresis. Confluent cultures of WI38 and AG 1518 cells were metabolically labeled for four hours in methionine-free DMEM supplemented with 1 mg/ml bovine serum albumin (BSA) and 0.15 mCi/ml Translabel (ICN, Costa Mesa, CA). Cells were lysed in 0.5% DOC, 0.5% Triton X-100, 150 mM NaCl, 20 mM Hepes, pH 7.5, 5μg/ml aprotinin, 5 μg/ml leupeptin, 1 mM benzamidine, 1 mM DTT (lysis buffer) and lysates were centrifuged at 15,000 x g for 15 minutes. Lysates corresponding to approximately 2 x 10^6 cells were then incubated with 20 μl of anti-CSH-243 or anti-CSH-243. After incubation for four hours at 4° C, 50 μl of a 1:1 Protein-A-Sepharose slurry was added to bind the protein/antibody complexes and incubation continued for 60 minutes. Immune complexes adsorbed to the Protein-A-Sepharose were collected by centrifugation and washed three times in 1% Triton X-100, 150 mM NaCl, 20 mM Hepes, pH 7.5, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 1 mM benzamidine, 1 mM DTT (washing buffer) and once in 20 mM Tris, pH 7.5. Samples were eluted from the resin by incubation at 95° C for 3 minutes in reducing SDS-sample buffer and analyzed by SDS-gel electrophoresis on 7% gels, followed by fluorography.

[0051] In both WI38 and AG 1518 cells, a protein of 180 kD was recognized specifically by the unblocked antisera. Anti-CSH-243 antisera immunoprecipitation with WI38 cell lysate also yielded significantly higher amounts (approximately 10 to 20 fold higher) of activity than precipitations with pre-immune serum or antiserum that had been previously incubated with 200 μg/ml of peptide-conjugate.

[0052] It appears that huDEP-1 is a phosphoprotein *in vivo* because the fact that the anti-CSH-243 antibody was capable of immunoprecipitating a 180 kD [^{32}P]-labeled protein from a cell lysate of WI38 cells which had been metabolically labelled with [^{32}P]-inorganic phosphate.

Example 6

Cell Density-Dependent Expression and Activity of huDEP-1.

[0053] WI38 cell lysates from sparse (less than 7,000 cells /cm^2) or dense (more than 25,000 cells/cm^2) cultures were compared for levels of expressed huDEP-1 protein by immunoblotting with anti-CSH-243 antibody as described in Example 4. A dramatic, ten- to twenty-fold increase in huDEP-1 expression was detected in dense cell cultures as shown in Figure 2. Since 3 μg of total cell lysate from more confluent culture gave a relatively strong signal, and 15 μg of lysates from sparse cultures were below detection, it was estimated that at least 10-fold higher amounts of huDEP-1 are present in cells from dense cultures. Similar results were obtained with anti-CSH-241. When the amounts of PTP1B in cell lysates from sparse and dense cells were compared using an anti-PTP1B monoclonal antibody FG6 (Oncogene Science, Uniondale, NY), no difference was observed. The observed effects on huDEP-1 expression are not restricted to WI38 cells as similar results were obtained in AG 1518 cells.

[0054] In order to determin if enzyme activity was also regulated by a density-dependent mechanism, huDEP-1 and PTP1B immune complexes and total cell lysates from both sparse and dense WI38 and AG 1518 cell cultures

were also analyzed for phosphatase activity using the PTP assay. For immune complex PTP assays, 60 μg of cell lysate were immunoprecipitated with 20 μl of anti-CSH-243 antisera (with or without pretreatment with antigen) or preimmune serum bound to 25 μl of Protein-A S pharos . After incubation overnight at 4° C, immune complexes were washed thr e tim s in washing buff r and once in 25 mM imidazole, pH 7.2, 0.5 mg/ml BSA, 1 mM DTT (assay buffer). Protein-A-S pharose immun complex s w r then suspended in 150 μl of assay buffer and assayed for PTP activity as triplicates. Assays were p rformed for 6 minutes at 30° C in a total volum of 60 μl using 3 μM [^{32}P-Tyr] RCM lysozym as substrate [Flint, *et al., supra*].

[0055] In agreement with the increased huDEP-1 protein expression demonstrated in the immunoblotting experiments, huDEP-1 enzyme activity also increased in the dense cell cultures. The observed increase in activity in huDEP-1/CSH-243 immunoprecipitates from dense cultures (approximately two-to three-fold) was not as great as the observed increase in protein expression in dense cultures, most likely due to incomplete precipitation of all of the PTP using anti-CSH-243 antisera. No difference was observed in activity of PTP1B/FG6 immunoprecipitates or total cell lysates from sparse and dense cell cultures.

[0056] Finally, to investigate the kinetics of the density-dependent upregulation of huDEP-1 expression, lysates of WI38 and AG 1518 cells at intermediate cell densities were included in the immunoblotting analysis. The highest expression was found in cells at saturation density, however, at intermediate densities an increase in expression with respect to sparse cell cultures was also observed. Thus, the upregulation of huDEP-1 expression appears to be initiated prior to saturation density and not a result of growth arrest.

[0057] While the precise mechanism by which huDEP-1 expression is induced remains unclear, the demonstration that expression was induced in two distinct cell lines as cells approach confluence suggests involvement of huDEP-1 in promoting net dephosphorylation of proteins, countering the effects of growth promoting PTK activity. This possibility, in combination with the broad distribution of huDEP-1 expression, suggests that huDEP-1 may be involved in a general mechanism for contact inhibition of cell growth.

Example 7

Identification of DEP-1 Ligands

[0058] The possibility that DEP-1 functions as an adhesion molecule will be tested using the Sf9 cell system [Brady-Kalnay, *et al., J. Cell Biol. 122*:961-972 (1993)] following transfection with DEP-1 cDNA. In addition to studies following transient expression, stable cell lines overexpressing DEP-1 will be generated.

[0059] If DEP-1 functions as an adhesion molecule, the extracellular counterreceptor(s) will be identified. One possibility is that, like PTPμ, DEP-1 binding is homophilic, where one DEP-1 molecule binds another DEP-1 molecule on an adjacent cell. Alternatively, DEP-1 specifically recognize a non-DEP-1 molecule in a heterophilic binding mechanism.

[0060] In addition, a number of deletion and site-directed mutagenesis strategies well known in the art will be applied to identify the important segments in the protein that confer binding specificity. Analysis of 2D gels of proteins that react with anti-phosphotyrosine antibodies, for example monoclonal antibody 4G10 (UBI, Lake Placid,NY), will be used to initiate studies as to the effect on activity of engagement of the extracellular segment of the PTP in either homophilic binding interactions or antibody binding.

[0061] Use of "epitope" library technology [Scott and Smith, *Science* 249:386-390 (1990)] will be employed to identify peptide sequences that interact with DEP-1. This approach will prove particularly useful in the search for ligands for DEP-1 whose extracellular segment, comprising multiple FNIII repeats, may bind low M_r factors.

[0062] Protein:protein interactions have previously been reported for FNIII sequences and specific binding proteins, and this information will be utilized in several approaches to identify proteins which specifically interact with the extracellular domain of DEP-1. Specifically, protein:protein interactions will be investigated in cell "panning" experiments [Seed and Aruffo, *Proc.Natl.Acad.Sci. (USA) 84*:3365-3369 (1987)], gel overlay assays [Hirsch, *et al., J.Biol.Chem. 267*:2131-2134 (1992); Carr and Scott, *Trends in Biochemical Sci.* 17:246-249 (1992)], band shift analysis [Carr, *et al., J.Biol.Chem.* 267:13376-13382 (1992)], affinity chromatography, screening of expression libraries [Young and Davis, *Proc.Natl.Acad.Sci. (USA) 80*:1194-1198 (1983)], etc.

Example 8

Identification of Modulators/Substrates of DEP-1

[0063] Potential substrates of predicted physiological relevance will be t sted for activity against the catalytic domain *in vitro.*

[0064] In addition, y ast scr ning syst ms [Fields and Song, *Nature* 340:245-246 (1989); Yang, *et al., Science 257*: 6810682 (1992); Vojt k, *et al., Cell 74*:205-214 (1993)] will be utiliz d, particularly with reference to co-expression with

a protein tyrosine kinas , for exampl , v-src r c-src, to isolate proteins with th capacity to regulat DEP-1 activity.

[0065] In a further attempt to identify substrates for DEP-1, a mutant form in which the cysteinyl residues of th activ center has been replac d by serine will be expressed. Recent studies suggest that substrates bind to and r main compl xed with th inactiv phosphatase. The mutant PTP is capable of binding substrate molecul s but traps them in a "dead end" complex that can b isolat d by standard immunoprecipitation techniques [Sun, *et al.*, *Cell 75*:487-493 (1993)]. Potential substrates may be co-immunoprecipitated with the mutant PTP from ^{35}S-label d c lls. Alternatively, wild-type, or native, DEP-1 enzyme may be utiliz d in this technique. Initial studies in this direction may make use f chimeric molecules, for which antibodies to the extracellular growth factor binding segment are commercially available, while antibodies are raised to the bona fide DEP-1 sequences.

Example 9

Characterization of the Genomic DEP-1 Gene

[0066] Isolation of the cDNA sequences for DEP-1 will permit the isolation and purification of the corresponding genomic sequences for DEP-1. In preliminary work, it has been demonstrated that huDEP-1 mapped to human chromosome 11p, band 11.2 or the interface of 11.2 and 11.3. Isolation of these genomic DEP-1 sequences will permit the identification of putative regulatory sequences for DEP-1 transcription, and presumably identification of *trans*-acting transcriptional modulators of DEP-1 expression. In addition, isolation and purification of the human genomic clone will permit screening of libraries in other species to determine if homologous counterparts exist in the species. Identification of a homologous counterpart in mice will be of particular importance because of the possibility of generating a knockout strain. Mouse strains which do not express a particular protein are of considerable importance in that they permit determination of indications associated with absence of the protein in a living animal.

SEQUENCE LISTING

[0067]

(1) GENERAL INFORMATION:

(i) APPLICANTS: Tonks, Nicholas K. and Östman, Arne
(ii) TITLE OF INVENTION: Density Enhanced Protein Tyrosine Phosphatase
(iii) NUMBER OF SEQUENCES: 6
(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
(B) STREET: 233 South Wacker Drive, Suite 6300
(C) CITY: Chicago
(D) STATE: Illinois
(E) COUNTRY: United States of America
(F) ZIP: 60606

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Borun, Michael F.
(B) REGISTRATION NUMBER: 25,447

(C) REFERENCE/DOCKET NUMBER: 27866/31954

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 312-474-6300
(B) TELEFAX: 312-474-0448

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5117 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 350..4364

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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    1250                1255                1260

Val Asp Val Tyr Gly Ile Val Tyr Asp Leu Arg Met His Arg Pro Leu
1265                1270                1275                1280

Met Val Gln Thr Glu Asp Gln Tyr Val Phe Leu Asn Gln Cys Val Leu
                1285                1290                1295

Asp Ile Val Arg Ser Gln Lys Asp Ser Lys Val Asp Leu Ile Tyr Gln
            1300                1305                1310

Asn Thr Thr Ala Met Thr Ile Tyr Glu Asn Leu Ala Pro Val Thr Thr
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Phe Gly Lys Thr Asn Gly Tyr Ile Ala
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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1               5
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: singl
(D) TOPOLOGY: lin ar

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AARTGYGCNC ARTAYTGGCC 20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE

(D) OTHER INFORMATION: /note= "Base designated N at postion 6 is Inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCDATNCCNG CRCTRCARTG 20

Claims

1. An isolated density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide of SEQ ID NO:2 or a variant thereof, wherein said variant is selected from:

(a) a human allelic variant or a variant from a heterologous species;

(b) a fragment thereof wherein:

(1) the fragment can be recognised by an antibody that is specific for a Type III receptor-like density enhanced protein tyrosine phosphatase, or

(2) at least one biological activity of a Type III receptor-like density enhanced phosphatase is retained or enhanced; said biological activity selected from

(i) binding to a substrate, ligand or counter-receptor of a Type III receptor-like density enhanced protein tyrosine phosphatase,

(ii) enzymatic activity of a Type III receptor-like density enhanced protein tyrosine phosphatase, or

(iii) signal transducti n activity f a Type III receptor-like d nsity nhanc d protein tyrosin phosphatase;

(c) a variant thereof in which one amino acid is replaced and wherein

(1) the variant can be recognised by an antibody that is specific for a Type III receptor-like density enhanced protein tyrosine phosphatase, or

(2) at least one biological activity of a Type III receptor-like density enhanced phosphatase is retained or enhanced; said biological activity selected from

(i) binding to a substrate, ligand or counter-receptor of a Type III receptor-like density enhanced protein tyrosine phosphatase,

(ii) enzymatic activity of a Type III receptor-like density enhanced protein tyrosine phosphatase, or

(iii) signal transduction activity of a Type III receptor-like density enhanced protein tyrosine phosphatase; or

(3) a specific ligand/receptor binding or signalling function is disabled; or

(d) a mutant thereof in which a cysteine residue in the catalytic domain is substituted with a serine residue.

2. A density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide or variant of claim 1 in completely glycosylated, partially glycosylated or non-glycosylated form.

3. An isolated polynucleotide encoding a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide or variant of claim 1 or 2.

4. The polynucleotide according to Claim 3 which is a DNA.

5. The DNA of Claim 4 which is selected from the group consisting of cDNA, genomic DNA, partially chemically synthesized DNA, and wholly chemically synthesized DNA.

6. The DNA of Claim 4 comprising a huDEP-1 protein coding sequence as set forth in SEQ ID NO:1.

7. The DNA of Claim 4 further comprising regulatory DNA sequences which direct transcription of the DNA.

8. A purified and isolated polynucleotide selected from the group consisting of:

(a) the DNA sequence set out in SEQ ID NO:1, and

(b) a DNA molecule which hybridizes under stringent conditions to the protein coding portion of the DNA of (a).

9. A DNA expression construct comprising the DNA of Claim 7.

10. A host cell transformed or transfected with the DNA of Claim 4.

11. A method for producing a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide or variant thereof comprising the steps of growing the host cell according to Claim 10 in a suitable medium and isolating the polypeptide or variant from the host cell or the medium of its growth.

12. An isolated antibody capable of specifically binding to a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide or variant of claim 1 or 2.

13. An antibody according to Claim 12 which is a monoclonal antibody.

14. An anti-idiotype antibody specific for the monoclonal antibody of Claim 13.

15. A hybridoma cell line producing the antibody of Claim 13 or 14.

16. A method for isolating a polynucleotide encoding a polypeptide that binds to a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide or variant of claim 1 or 2, comprising the steps of:

(a) transforming or transfecting host cells with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA-binding domain and an activating domain;

(b) expressing in the host cells a first hybrid DNA sequence encoding a first fusion of part or all of a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide or variant of claim 1 or 2 and either the DNA-binding domain or the activating domain of the transcription factor;

(c) expressing in the host cells a library of second hybrid DNA sequences encoding second fusions of part or all of putative density enhanced Type III receptor-like protein tyrosine phosphatase binding polypeptides and either the activating domain or DNA binding domain of the transcription factor which is not incorporated in the first fusion;

(d) transforming or transfecting the host cells with a DNA construct comprising a protein tyrosine kinase gene;

(e) detecting binding of density enhanced Type III receptor-like protein tyrosine phosphatase binding polypeptide(s) to the phosphatase polypeptide in the host cell by detecting the production of reporter gene product in the host cell(s); and

(f) isolating the second hybrid DNA sequences encoding the phosphatase binding polypeptide or variant from the host cell(s).

17. The method of Claim 16 wherein the promoter is the beta-galactosidase promoter, the DNA-binding domain is the *lexA* DNA-binding domain, the activating domain is the GAL4 transactivation domain, the reporter gene is the *lacZ* gene and the host cells are yeast host cells.

18. The method according to Claim 16 wherein the density enhanced Type III receptor-like protein tyrosine phosphatase is a catalytically inactive variant of huDEP-1 capable of binding huDEP-1 substrate.

19. A method for detecting proteins which bind to a density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide isoform of claim 1 or 2 comprising the steps of:

(a) transforming or transfecting host cells with a hybrid DNA sequence encoding a fusion between a putative phosphatase binding protein and a ligand capable of high affinity binding to a specific counterreceptor;

(b) expressing the hybrid DNA sequence in the host cells under appropriate conditions;

(c) immobilizing fusion protein from the host cells by exposing the fusion protein to the specific counterreceptor in immobilized form;

(d) contacting the density enhanced Type III receptor-like protein tyrosine phosphatase polypeptide with the immobilized fusion protein; and

(e) detecting the phosphatase polypeptide bound to the fusion protein using a reagent specific for the phosphatase polypeptide.

20. The method of Claim 19 wherein the ligand is glutathione-S-transferase and the counterreceptor is glutathione.

21. The method of Claim 19 wherein the ligand is hemagglutinin and the counterreceptor is a hemagglutinin-specific antibody.

22. The method of Claim 19 wherein the ligand is a polyhistidine and the counterreceptor is nickel.

23. The method of Claim 19 wherein the ligand is maltose-binding protein and the counterreceptor is amylose.

24. An antibody of any one of claims 12 to 14 for use as a therapeutic or prophylactic agent.

25. Use of an antibody of any one of claims 12 to 14 for the manufacture of a medicament for the treatment of malignant cell growth.

Patentansprüche

1. Ein isoliertes, Dichte-stimuliertes, Typ-III-rezeptorartiges Proteintyrosinphosphatase-Polypeptid der SEQ ID NO: 2 oder eine Variante davon, wobei die Variante ausgewählt ist aus:

a) einer menschlichen Allel-Variante oder einer Variante von einer heterologen Spezies;

b) einem Fragment davon, wobei:

(1) das Fragment von einem Antikörper erkannt werden kann, der für eine Typ-III-rezeptorartige, Dichte-stimulierte Proteintyrosinphosphatase spezifisch ist, oder

(2) wenigstens eine biologische Aktivität einer Typ-III-rezeptorartigen, Dichte-stimulierten Phosphatase beibehalten oder verstärkt ist; wobei die biologische Aktivität ausgewählt ist aus

(i) Bindung an ein Substrat, Ligand oder Gegenrezeptor einer Typ-III- rezeptorartigen, Dichte-stimulierten Proteintyrosinphosphatase,

(ii) enzymatischer Aktivität einer Typ-III-rezeptorartigen, Dichte-stimulierten Proteintyrosinphosphatase, oder

(iii) Signaltransduktionsaktivität einer Typ-III-rezeptorartigen, Dichte-stimulierten Proteintyrosinphosphatase;

c) einer Variante davon, bei der eine Aminosäure ersetzt ist, und wobei

(1) die Variante von einem Antikörper erkannt werden kann, der für eine Typ-III-rezeptorartige Dichte-stimulierte Proteintyrosinphosphatase spezifisch ist, oder
(2) wenigstens eine biologische Aktivität einer Typ-III-rezeptorartigen, Dichte-stimulierten Phosphatase beibehalten oder verstärkt ist; wobei die biologische Aktivität ausgewählt ist aus
(i) Bindung an ein Substrat, Ligand oder Gegenrezeptor einer Typ-III-rezeptorartigen, Dichte-stimulierten Proteintyrosinphosphatase,

(ii) enzymatischer Aktivität einer Typ-III-rezeptorartigen, Dichte-stimulierten Proteintyrosinphosphatase, oder

(iii) Signaltransduktionsaktivität einer Typ-III-rezeptorartigen, Dichte-stimulierten Proteintyrosinphosphatase; oder

(3) eine spezifische Ligand/Rezeptor-Bindung oder Signalfunktion außer Kraft gesetzt ist; oder

d) einer Mutante davon, bei der ein Cysteinrest in der katalytischen Domäne gegen einen Serinrest ausgetauscht ist.

2. Dichte-stimuliertes, Typ-III-rezeptorartiges Proteintyrosinphosphatase-Polypeptid oder Variante nach Anspruch 1 in vollständig glykosylierter, teilweise glykosylierter oder nicht-glykosylierter Form.

3. Ein isoliertes Polynukleotid, das für ein Dichte-stimuliertes, Typ-III-rezeptorartiges Proteintyrosinphosphatase-Polypeptid oder Variante nach Anspruch 1 oder 2 kodiert.

4. Polynukleotid nach Anspruch 3, das eine DNA ist.

5. DNA nach Anspruch 4, die ausgewählt ist aus der Gruppe, bestehend aus cDNA, genomischer DNA, teilweise chemisch synthetisierter DNA und vollständig chemisch synthetisierter DNA.

6. DNA nach Anspruch 4, umfassend eine huDEP-1-Protein-kodierende Sequenz, wie dargestellt in SEQ ID NO: 1.

7. DNA nach Anspruch 4, weiterhin umfassend regulatorische DNA-Sequenzen, die die Transkription der DNA steuern.

8. Ein aufgereinigtes und isoliertes Polynukleotid, ausgewählt aus der Gruppe, bestehend aus:

   (a) der DNA-Sequenz, die in SEQ ID NO: 1 dargestellt ist, und

   (b) einem DNA-Molekül, das unter stringenten Bedingungen an den Protein-kodierenden Teil der DNA von (a) hybridisiert.

9. Ein DNA-Expressionskonstrukt, umfassend die DNA nach Anspruch 7.

10. Eine Wirtszelle, transformiert oder transfiziert mit der DNA nach Anspruch 4.

11. Ein Verfahren zum Herstellen eines Dichte-stimulierten, Typ-III-rezeptorartigen Proteintyrosinphosphatase-Polypeptids oder einer Variante davon, umfassend die Schritte: Wachsenlassen der Wirtszelle nach Anspruch 10 in einem geeigneten Medium und Isolieren des Polypeptids oder der Variante aus der Wirtszelle oder ihrem Wachstumsmedium.

12. Ein isolierter Antikörper, der in der Lage ist, spezifisch an ein Dichte-stimuliertes, Typ-III-rezeptorartiges Proteintyrosinphosphatase-Polypeptid oder eine Variante nach Anspruch 1 oder 2 zu binden.

13. Antikörper nach Anspruch 12, der ein monoklonaler Antikörper ist.

14. Ein Anti-Idiotyp-Antikörper, der für den monoklonalen Antikörper nach Anspruch 13 spezifisch ist.

15. Eine Hybridoma-Zellinie, die den Antikörper nach Anspruch 13 oder 14 erzeugt.

16. Ein Verfahren zum Isolieren eines Polynukleotids, das für ein Polypeptid kodiert, das an ein Dichte-stimuliertes, Typ-III-rezeptorartiges Proteintyrosinphosphatase-Polypeptid oder eine Variante nach Anspruch 1 oder 2 bindet, umfassend die Schritte:

   (a) Transformieren oder Transfizieren von Wirtszellen mit einem DNA-Konstrukt, umfassend ein Reportergen unter der Kontrolle eines Promoters, reguliert durch einen Transkriptionsfaktor mit einer DNA-Bindungsdomäne und einer Aktivierungsdomäne;

   (b) Exprimieren einer ersten Hybrid-DNA-Sequenz, die für eine erste Fusion eines Teils oder eines ganzen Dichte-stimulierten, Typ-III-rezeptorartigen Proteintyrosinphosphatase-Polypeptids oder einer Variante nach Anspruch 1 oder 2 und entweder der DNA-Bindungsdomäne oder der Aktivierungsdomäne des Transkriptionsfaktors kodiert, in den Wirtszellen;

   (c) Exprimieren einer Bibliothek von zweiten Hybrid-DNA-Sequenzen, die für zweite Fusionen eines Teils oder ganzer mutmaßlicher Dichte-stimulierter, Typ-III-rezeptorartiger Proteintyrosinphosphatase-Bindungspolypeptide und entweder der Aktivierungsdomäne oder DNA-Bindungsdomäne des Transkriptionsfaktors kodieren, die nicht in die erste Fusion eingebaut ist, in den Wirtszellen;

   (d) Transformieren oder Transfizieren der Wirtszellen mit einem DNA-Konstrukt, umfassend ein Proteintyrosinkinasegen;

   (e) Nachweisen der Bindung des (der) Dichte-stimulierten, Typ-III-rezeptorartigen Proteintyrosinphosphatase-Bindungspolypeptids(polypeptide) an das Phosphatasepolypeptid in der Wirtszelle durch Nachweis der Produktion des Reportergenprodukts in der (den) Wirtszelle(n); und

   (f) Isolieren der zweiten Hybrid-DNA-Sequenzen, die für das Phosphatase-Bindungspolypeptid oder die Variante kodieren, aus der (den) Wirtszelle(n).

17. Verfahren nach Anspruch 16, wobei der Promoter der beta-Galactosidase-Promot r ist, di DNA-Bindungsdomäne die *lexA*-DNA-Bindungsdomäne ist, die Aktivierungsdomäne di GAL4-Transaktivierungsdomäne ist, das Repor- t rgen das *lacZ*-Gen lst, und die Wirtszellen Hefe-Wirtszellen sind.

18. Verfahren nach Anspruch 16, wobei die Dichte-stimuli rte, Typ-III-rezeptorartige Prot intyr sinphosphatase eine katalytisch inaktive Variant von huDEP-1 ist, die in der Lage ist, huDEP-1-Substrat zu binden.

19. Verfahren zum Nachweis von Proteinen, die an eine Dichte-stimulierte Typ-III-rezeptorartige Proteintyrosinphosphatase-Polypeptid-Isoform nach Anspruch 1 oder 2 binden, umfassend die Schritte:

(a) Transformieren oder Transfizieren von Wirtszellen mit einer Hybrid-DNA-Sequenz, die für eine Fusion zwischen einem mutmaßlichen Phosphatase-Bindungsprotein und einem Liganden kodiert, der in der Lage ist, an einen spezifischen Gegenrezeptor mit hoher Affinität zu binden.

(b) Exprimieren der Hybrid-DNA-Sequenz in den Wirtszellen unter geeigneten Bedingungen;

(c) Immobilisieren des Fusionsproteins aus den Wirtszellen durch Aussetzen des Fusionsproteins gegenüber dem spezifischen Gegenrezeptor in immobilisierter Form;

(d) In-Kontakt-Bringen des Dichte-stimulierten, Typ-III-rezeptorartigen Proteintyrosinphosphatase-Polypeptids mit dem immobilisierten Fusionsprotein; und

(e) Nachweisen des an das Fusionsprotein gebundenen Phosphatase-Polypeptids unter Verwendung eines Reagenz, das für das Phosphatase-Polypeptid spezifisch ist.

20. Verfahren nach Anspruch 19, wobei der Ligand Glutathion-S-Transferase lst, und der Gegenrezeptor Glutathion ist.

21. Verfahren nach Anspruch 19, wobei der Ligand Hämagglutinin ist und der Gegenrezeptor ein Hämagglutinin-spezifischer Antikörper ist.

22. Verfahren nach Anspruch 19, wobei der Ligand ein Polyhistidin ist und der Gegenrezeptor Nickel ist.

23. Verfahren nach Anspruch 19, wobei der Ligand Maltose-Bindungsprotein ist und der Gegenrezeptor Amylose ist.

24. Ein Antikörper nach einem der Ansprüche 12 bis 14 zur Verwendung als ein therapeutisches oder prophylaktisches Mittel.

25. Verwendung eines Antikörpers nach einem der Ansprüche 12 bis 14 zur Herstellung eines Medikaments zur Behandlung malignen Zellwachstums.

Revendications

1. polypeptide protéine-tyrosine-phosphatase analogue au récepteur de Type III stimulé par la densité, isolé, répondant à la SEQ ID NO:2 ou un de ses variants, ledit variant étant choisi entre :

(a) un variant allélique humain ou un variant provenant d'une espèce hétérologue ;
(b) un de ses fragments, où :

(1) le fragment peut être reconnu par un anticorps qui est spécifique d'une protéine-tyrosine-phosphatase stimulée par la densité, analogue au récepteur de Type III, ou
(2) au moins une activité biologique d'une phosphatase stimulée par la densité, analogue au récepteur de Type III, est maintenue ou amplifiée; ladite activité biologique étant choisie entre

(i) la liaison à un substrat, ligand ou contre-récept ur d'une protéine-tyrosine-phosphatase stimulée par la densité, analogue au récepteur de Typ III,
(ii) l'activité enzymatique d'une protéine-tyrosine-phosphatase stimulée par la densité, analogue au récepteur de Type III, ou

(iii) une activité de transduction de signal d'un protéine-tyrosine-phosphatase stimulée par la densité, analogue au récepteur de Type III.

(c) un de ses variants dans lequel un amino-acide est remplacé et

(1) le variant peut être reconnu par un anticorps qui est spécifique d'un protéine-tyrosin-phosphatase stimulée par la densité, analogue au récepteur de Type III, ou
(2) au moins une activité biologique d'une phosphatase stimulée par la densité, analogue au récepteur de Type III, est maintenue ou amplifiée ; ladite activité biologique étant choisie entre

(i) la liaison à un substrat, ligand ou contre-récepteur d'une protéine-tyrosine-phosphatase stimulée par la densité, analogue au récepteur de Type III, ou
(ii) l'activité enzymatique d'une protéine-tyrosine-phosphatase stimulée par la densité, analogue au récepteur de Type III, ou
(iii) une activité de transduction de signal d'une protéine-tyrosine-phoephatase stimulée par la densité, analogue au récepteur de Type III ; ou

(3) une liaison ligand/récepteur spécifique ou une fonction de transmission de signal est mise hors service ; ou

(d) un de ses mutants dans lequel un résidu cystéine dans le domaine catalytique est remplace par un résidu sérine.

2. Polypeptide protéine-tyrosine-phosphatase analogue au récepteur de Type III, stimulé par la densité ou variant, suivant la revendication 1, sous une forme totalement glycosylée, partiellement glycosylée ou non-glycosylée.

3. Polynucléotide isolé codant pour un polypeptide protéine-tyrosine-phosphatase analogue au récepteur de Type III, stimulé par la densité, ou un variant suivant la revendication 1 ou 2.

4. Polynucléotide suivant la revendication 3, qui est un ADN.

5. ADN suivant la revendication 4, qui est choisi dans le groupe consistant en un ADNc, un ADN génomique, un ADN partiellement synthétisé chimiquement et un ADN totalement synthétisé chimiquement.

6. ADN suivant la revendication 4, comprenant une séquence codant pour la protéine huDEP-1 telle que définie dans la SEQ ID NO:1.

7. ADN suivant la revendication 4, comprenant en outre des séquences d'ADN régulatrices qui dirigent la transcription de l'ADN.

8. Polynucléotide purifié isolé choisi dans le groupe consistant en :

(a) la séquence d'ADN définie dans la SEQ ID NO:1, et
(b) une molécule d'ADN qui s'hybride dans des conditions drastiques à la portion codant la protéine de l'ADN de (a).

9. Produit d'assemblage d'expression d'ADN comprenant l'ADN suivant la revendication 7.

10. Cellule-hôte transformée ou transfectée avec l'ADN suivant la revendication 4.

11. Procédé pour la production d'un polypeptide protéine-tyrosine-phosphatase analogue au récepteur de Type III, stimulé par la densité, ou d'un de ses variants, comprenant les étapes consistant à cultiver la cellule-hôte suivant la revendication 10 dans un milieu convenable et isoler le polypeptide ou variant de la cellule-hôte ou de son milieu de croissance.

12. Anticorps isolé capable de se lier spécifiquement à un polypeptide protéine-tyrosin-phosphatase analogue au récepteur de Type III, stimulé par la densité, ou variant suivant la revendication 1 ou 2.

13. Anticorps suivant la revendication 12, qui est un anticorps monoclonal.

14. Anticorps anti-idiotype spécifique de l'anticorps monoclonal suivant la revendication 13.

15. Ligné de cellules d'hybridome produisant l'anticorps suivant la revendication 13 ou 14.

16. Procédé pour isoler un polynucléotide codant pour un polyp ptide qui se lie à un polypeptide protéine-tyrosine-phosphatase analogue au récepteur de Type III, stimulé par la densité ou un variant suivant la revendication 1 ou 2, comprenant les étapes consistant :

(a) à transformer ou transfecter des cellules-hôtes avec un produit d'assemblage d'ADN comprenant un gène rapporteur sous le contrôle d'un promoteur régulé par un facteur de transcription ayant un domaine de liaison d'ADN et un domaine activateur ;
(b) à provoquer l'expression dans les cellules-hôtes d'une première séquence d'ADN hybride codant pour une première fusion d'une partie ou de la totalité d'un polypeptide protéine-tyrosine-phospnatase analogue au récepteur de Type III, stimulé par la densité ou un variant suivant la revendication 1 ou 2 et soit du domaine de liaison d'ADN, soit du domaine activateur du facteur de transcription ;
(c) à provoquer l'expression dans les cellules-hôtes d'une banque de secondes séquences d'ADN hybrides codant des secondes fusions d'une partie ou de la totalité de polypeptides putatifs de liaison de protéine-tyrosine-phosphatase analogue au récepteur de Type III, stimulé par la densité et soit du domaine activateur, soit du domaine de liaison d'ADN du facteur de transcription qui n'est pas incorporé à la première fusion ;
(d) à transformer ou transfecter les cellules-hôtes avec un produit d'assemblage d'ADN comprenant un gène de protéine-tyrosine-kinase;
(e) à détecter la liaison du ou des polypeptides de liaison de protéine-tyrosine-phosphatase analogue au récepteur de Type III, stimulé par la densité, au polypeptide phosphatase dans la cellule-hôte en détectant la production du produit de gêne rapporteur dans la ou les cellules-hôtes ; et
(e) à isoler les secondes séquences d'ADN hybrides codant pour le polypeptide de liaison de phosphatase ou un variant à partir de la ou des cellules-hôtes.

17. Procédé suivant la revendication 16, dans lequel le promoteur est le promoteur bêta-galactosidase, le domaine de liaison d'ADN est le domaine de liaison d'ADN *lexA*, le domaine activateur est le domaine de transactivation GAL4, le gène rapporteur est le gêne *lacZ* et les cellules-hôtes sont des cellules-hôtes de levures.

18. Procédé suivant la revendication 16, dans lequel la protéine-tyrosine-phosphatase analogue au récepteur de Type III, stimulée par la densité, est un variant catalytiquement inactif de huDEP-1 capable de se lier au substrat de huDEP-1.

19. Procédé pour détecter des protéines qui se lient à une isoforme du polypeptide protéine-tyrosine-phosphatase analogue au récepteur de Type III, stimulé par la densité, suivant la revendication 1 ou 2, comprenant les étapes consistant :

(a) à transformer ou transfecter des cellules-hôtes avec une séquence d'ADN hybride codant pour une fusion entre une protéine de liaison de phosphatase putative et un ligand apte à la liaison à forte affinité à un contre-récepteur spécifique ;
(b) à provoquer l'expression de la séquence d'ADN hybride dans les cellules-hôtes dans des conditions appropriées ;
(c) à immobiliser la protéine de fusion à partir des cellules-hôtes en exposant la protéine de fusion au contre-récepteur spécifique sous forme immobilisée ;
(d) à mettre en contact le polypeptide protéine-tyrosine-phosphatase analogue au récepteur de Type III stimulé par la densité, avec la protéine de fusion immobilisée ; et
(e) à détecter le polypeptide phosphatase lié à la protéine de fusion en utilisant un réactif spécifique du polypeptide phosphatase.

20. Procédé suivant la revendication 19, dans lequel le ligand est la glutathion-S-transférase et le contre-récepteur st le glutathion.

21. Procédé suivant la revendication 19, dans lequel le ligand est l'hémagglutinin et le contre-récepteur est un anticorps spécifique de l'hémagglutinine.

22. Procédé suivant la revendication 19, dans lequel le ligand est une polyhistidine et le contre-récepteur est le nickel.

23. Procédé suivant la revendication 19, dans lequel le ligand est la protéine de liaison, la protéine fixant le maltose et le contre-récepteur est l'amylose.

24. Anticorps suivant l'une quelconque des revendications 12 à 14, destiné à être utilisé comme agent thérapeutique ou prophylactique.

25. Utilisation d'un anticorps suivant l'une quelconque des revendications 12 à 14 pour la production d'un médicament destiné au traitement de la croissance de cellules malignes.

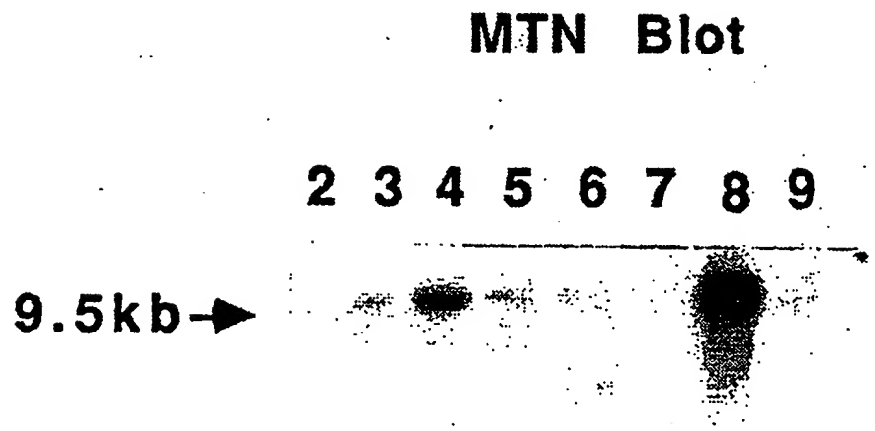

FIG. 1A

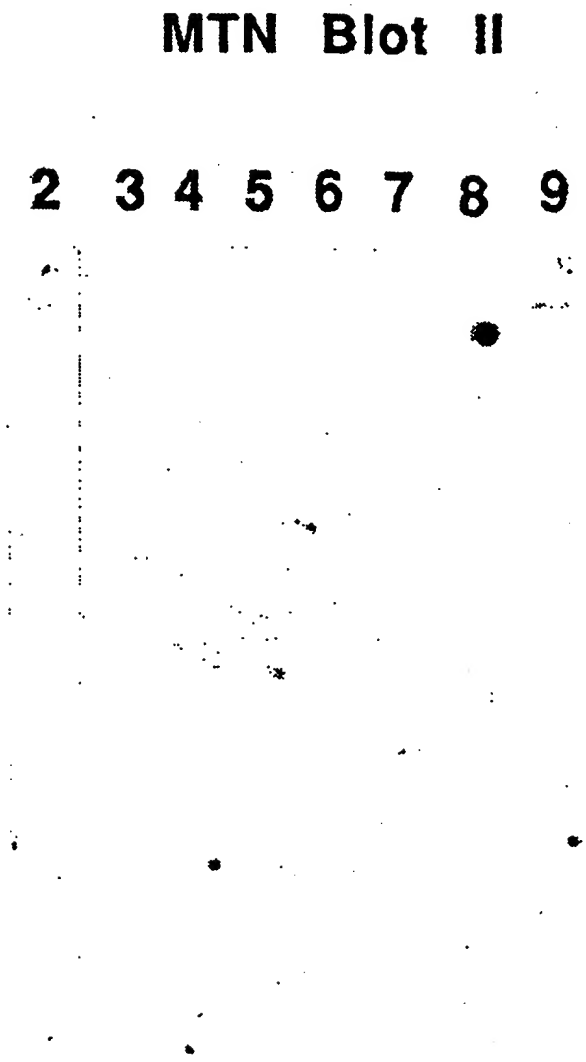

FIG. 1B

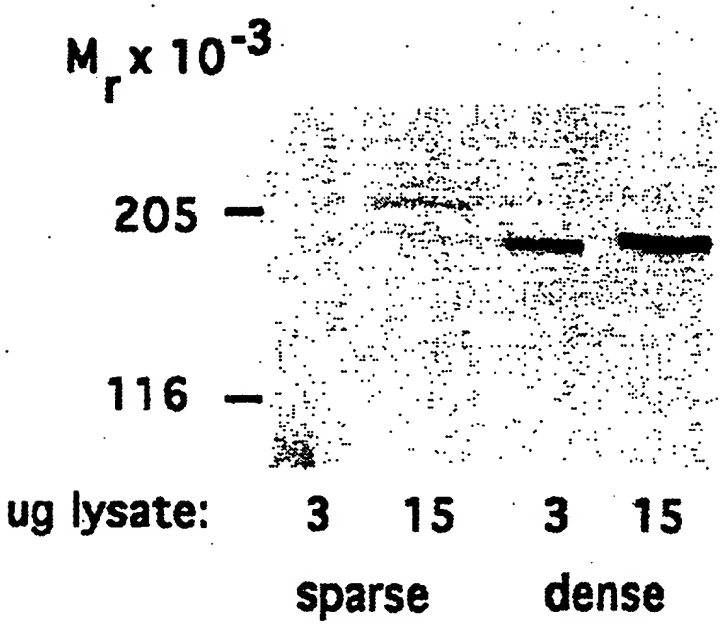

FIG. 2